



# PHYTOTOXIC EFFECT OF *TECTONA GRANDIS*(L.f.) LEAF EXTRACTS ON GROWTH AND DEVELOPMENTAL CHANGES OF *PENNISETUM GLAUCUM* (L.) R.BR. AND *ELEUSINE CORACANA* (Gaertn)

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## ABSTRACT

The present study investigates the growth and developmental changes of Pearl millet [*Pennisetum glaucum* (L.) R.Br.] and Ragi [*Eleusine coracana* (Gaertn.)] under pot culture experiments with different concentrations of aqueous leaf extracts (5, 10, 20, 30 and 50g/l) of Teak. Teak leaf extracts showed an inhibitory and stimulatory effects on germination, seedling length, biomass, pigments and biochemical constituents of pearl millet and ragi. The 5g/l concentration of extract stimulated the seed germination, seedling growth and biochemical constituents of pearl millet and ragi. The higher concentrations (10, 20, 30 and 50g/l) showed an inhibitory effects in all the parameters studied in the two economically important cereals. The degree of retardation was dependent on the concentration of the Teak leaf extracts. Root length, Shoot length, Biomass and Biochemical constituents of ragi seedlings were highly reduced at 50g/l concentrations of aqueous leaf extracts of Teak. Between pearl millet and ragi seedlings, more allelopathic influence was observed in ragi than pearl millet seedlings.

**KEY WORDS:** Allelopathy, extracts, Teak, Pearl millet, Ragi, cereals.

## INTRODUCTION:

Agroforestry is the integration of Agriculture and Forestry to increase the farm productivity and sustainability of farming systems (Fikreyesus *et al.*, 2011). Agroforestry systems make maximum use of the land. Every part of the land is considered suitable for useful plants. Emphasis is placed on perennial, multiple purpose crops that are planted once and yield benefits over a long period of time. Such benefits include construction materials, food for humans and animals, fuels, fibers, and shade. Trees in agroforestry systems also have an important uses such as holding the soil against erosion and improving soil fertility (by fixing nitrogen or bringing minerals from deep in the soil and depositing them by leaf-fall). Furthermore, well-designed systems of agroforestry maximize beneficial interactions of the crop plants while minimizing unfavorable interactions. The most common interaction is competition, which may be for light, water, or soil nutrient. The agroforestry plants remain productive for the farmers and generate continuous revenue, which is not the case when arable land is exclusively reforested. Agroforestry allows for the diversification of farm activity and makes better use of environmental resources.

Farooq *et al.* (2011a) and Bhadoria, (2011) stated that the allelochemicals are mostly secondary metabolites, which are produced as byproducts during different physiological processes in plants. Kurse *et al.* (2000) and Jabran and Farooq (2012) found that the important secondary metabolites identified as allelochemicals are phenols, alkaloids, flavonoids, terpenoids, hydroxamic acids, jasmonates, salicylates, carbonates and aminoacids. At higher concentrations, these allelochemicals may be used as natural pesticides (Farooq *et al.*, 2009c). Allelochemicals have great potential of nutrient cycling and nutrient regulation in agro-ecosystems. They offer an eco-friendly and sustainable way to manage the crop nutrient requirements. Breeding and biotechnology efforts can lead us to the development of genotypes having allelochemicals involved in solubilization, transformation, release, mobilization and uptake of essential nutrients. The production of allelochemicals is influenced by age of plant, type of stress, intensity of stress and ambient surroundings.

Plants use secondary metabolites as messenger under suboptimal conditions to trigger the defense mechanism. It starts the production of phytochemicals, hormones, biologically active secondary metabolites and variety of proteins necessary to defend the plant ultra structures from such hazards (Pedrol *et al.*, 2006). Under heat, drought or salinity stress, allelochemicals play a vital role in Reactive Oxygen Species (ROS) production initially and then activation of antioxidant defense system (Bogatek and Ginazdoeska, 2007). Adverse effects of abiotic stresses are due to abnormal biological, biochemical, morphological and physiological functions of plants. For instance, soil salinity induces the oxidative stress by the production of ROS causing reduction of photosynthetic electron chain (Waskiewicz *et al.*, 2013). Allelochemicals have direct as well as indirect effects on plants. Rizvi *et al.* (1992) stated that the direct action of secondary metabolites is function of different biochemical and physiological changes imparted in growth metabolism of plants. Allelopathy plays an important role in agricultural ecosystems and in the plants covers among the crop-crop, crop-weed and tree-crop covers. These interactions are detrimental and occasionally, are useful and gave attention to allelopathy in natural and agricultural ecosystems. Naseem *et al.* (2009) stated that allelopathy is recognized as appropriate potential technology to control weeds using chemicals released from decomposed various species of plant parts.

*Tectona grandis* L.f. belongs to the family Lamiaceae, mostly found in tropical region. Teak is a large, deciduous tree that occurs in mixed hardwood forests. It has small, fragrant white flowers and large papery leaves that are often hairy on the lower surface. It is sometimes known as the “Burmese teak”. It has a high potential of allelochemicals and also essential oils. Many studies have revealed that the allelopathic effects of Teak species and conformed the strong inhibitory effects of Teak extracts on some crops (Zhang and Shenglei, 2010; Leela and Arumugam, 2014). Leaf extract of Teak inhibited seed germination and reduced root and shoot lengths of cucumber and maximum inhibition was observed in higher concentrations of extract (Allolli and Narayanareddy, 2000).

Pearl millet is the most widely grown type of millet. It has been grown in Africa and the Indian subcontinent since prehistoric times. Ragi is an annual herbaceous plant widely grown as a cereal crop in the arid and semiarid areas in Africa and Asia. The present study was conducted to determine the influence of aqueous leaf leachates of Teak on seed germination, seedling growth, dry weight chl. a, chl. b, total chlorophyll, carotenoids, starch, protein and amino acid contents of pearl millet and ragi.

## MATERIALS AND METHODS:

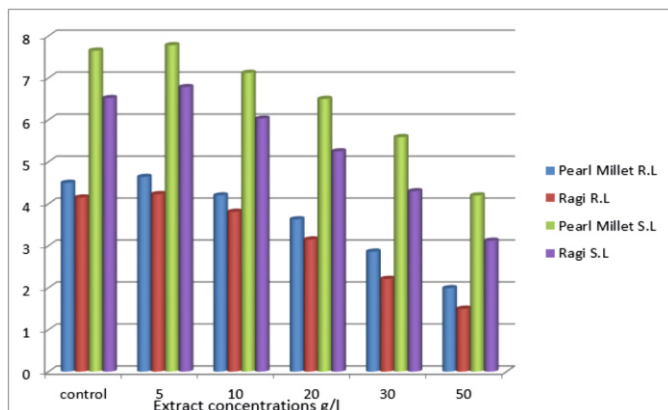
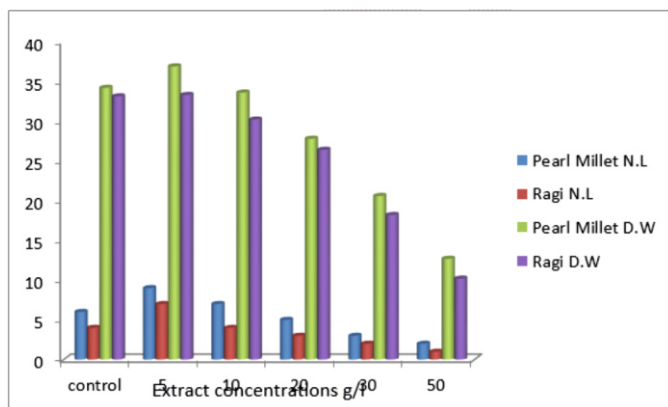
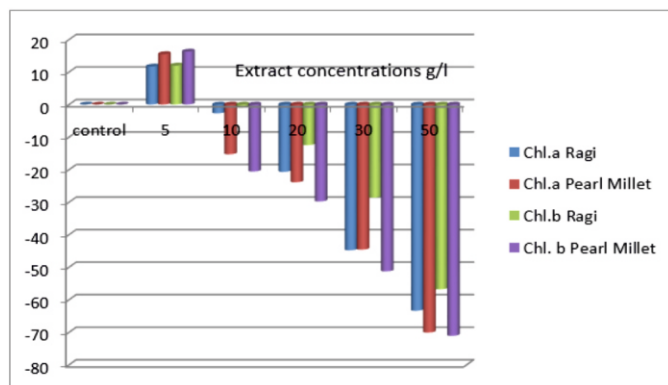
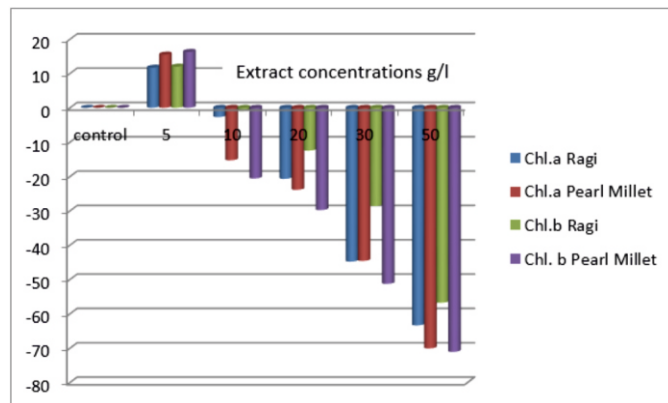
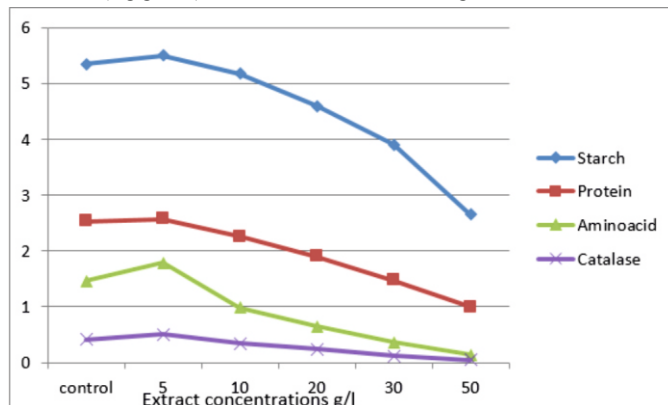
The fresh, mature and healthy leaves were collected from actively growing Teak plants were collected during the month of May from the social forestry of Cuddalore District of Annamalai Nagar (11.45°N 70.45°E) for the present study. The leaves were washed with distilled water thoroughly to remove the adherent dust particle, then dried for two weeks at room temperature and powdered with the help of powder wiley machine and stored at room temperature. 50 gram teak leaf powder soaked in 1000 ml distilled water for 48 hours. These extracts were filtered and filtrates were considered as 50g/l concentration. The same method was followed for 5, 10, 20 and 30 g/l Teak leaf extracts preparation. The obtained Teak leaf extracts was analyzed for phytochemical profiles by GC-MS. The freshly prepared extracts were used for the pot culture experiments.

Healthy uniform seeds of pearl millet and ragi seeds were collected from Tamil Nadu Agricultural University, Coimbatore. The seeds were pre-soaked in distilled water for overnight. Before germination, the seeds were surface sterilized with 0.1% HgCl<sub>2</sub> solution for 30 seconds and washed in distilled water thoroughly for several times to remove excess of chemical and dried on absorbent to eliminate fungal attack. Twenty five seeds each of pearl millet and ragi were sown in earthen pots (30×15cm) filled with garden soil having silt, humus and sand (pH-7.3, N-0.13, P-0.29, K-0.09 and OC-1.86%). Each pot was added with 200 ml of different concentrations of leaf extracts and control was treated with 200 ml of water. The experiment was conducted in completely randomized design with three replications. After 15 days of germination, the morphological and biochemical parameters were studied.

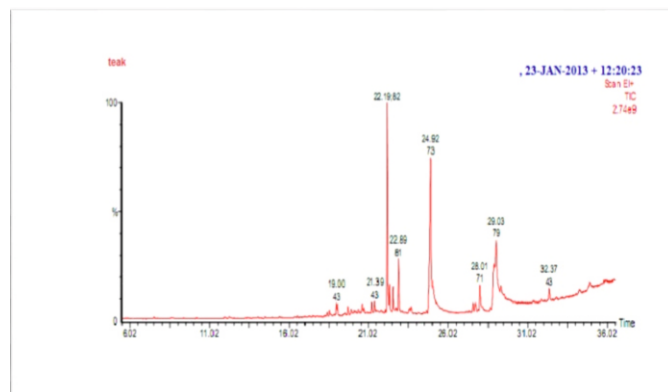
**Table - 1:** Allelopathic Influence of *Tectona grandis* leaf extracts on germination (%) of Pearlmillet and Ragi

Extracts Concentrations (g/l)	Pearlmillet	Ragi
control	98	98
5	100 (2.0)	100 (2.0)
10	96 (-2.0)	86 (-12.2)
20	82 (-16.3)	73 (-25.5)
30	68 (-30.6)	62 (-36.7)
50	47 (-52.0)	40 (-59.1)

Data in parentheses indicate % increase/decrease over control.

**Fig - 1 :** Allelopathic Influence of *Tectona grandis* leaf extracts on Root length (cm/seedling) and shoot length (cm/seedling) of Pearlmillet and Ragi**Fig - 2:** Allelopathic Influence of *Tectona grandis* leaf extracts on No. of leaves and Dry weight (mg/seedling) of Pearlmillet and Ragi**Fig - 3:** Allelopathic Influence of *Tectona grandis* leaf extracts on Chl. a and Chl. b (mg/g fr.wt) of Pearlmillet and Ragi**Fig- 4:** Allelopathic Influence of *Tectona grandis* leaf extracts on Total Chl. and carotenoid (mg/g fr.wt) content of Pearlmillet and Ragi**Fig -5:** Allelopathic Influence of *Tectona grandis* leaf extracts on Starch, Protein, Amino acid and Catalase (mg/g fr.wt) content of Pearl Millet Seedlings

#### GC-MS Spectrum of *Tectona grandis*

**Fig-6:** Allelopathic Influence of *Tectona grandis* leaf extracts on Starch, Protein, Amino acid and Catalase (mg/g fr.wt) content of Ragi Seedlings

#### RESULTS AND DISCUSSION:

The germination percentage of five different concentrations of extracts (5, 10, 20, 30 and 50%) and water (control) is shown in Table:1. The results revealed that the 50% concentration of extract strongly reduced the germination percentage of ragi and pearl millet (47% and 40%) compared to that of 5% extract (100% and 100%). All concentrations of *Teak* leaf extracts did not show the same degree of reducing nature of germination. At 5% extract concentration the germination percentage increased when compared to control. The inhibition of germination is dependent on the concentration of the extract which may be due to the entry of water soluble allelochemicals into the seed inhibiting the germination. During germination, biochemical changes take place, which provide the basic framework for subsequent growth and development. There were significant variations found to different treatments at all concentrations. Suseelamma and Venkataraju, (1994) found that the *Digera muricata* leaf extracts reduced the germination and seedling growth of groundnut. Mohamadi and Rajaie, (2009) reported sorghum seeds germination was significantly reduced when treated with *Eucalyptus camaldulensis*. Mubarak et al. (2009) ; Phiri (2010) reported *Moringa oleifera*, *Khaya senegalensis* and *Albizia lebek* leaf extracts found to have no significant effects on seed germination of sorghum.

The Teak leaf extract significantly reduced the root length, shoot length and dry weight of ragi and pearl millet at 50% extract treatment when compared to the control (Fig - 1). But at 5% concentration of extract treatment, the test crops showed the promotory effects on root length, shoot length and dry weight over control. The highest reduction percentage of shoot length (63.4%) was recorded in ragi at 50% extract treatment. Seedling growth of ragi and pearl millet reduced progressively with increasing concentrations of extract. The more reduction of dry weight of ragi and pearl millet at 50% leachate concentration was 69.6% and 75.1% respectively. The results of present study were similar to those of Malik (2004), El-Khawas and Shehata (2005), Yamagushi *et al.* (2011), Mahmood Dejam *et al.* (2014) who have studied allelopathic effect of *E. globulus* leaf extract on germination and seedling growth of some vegetable and crop plants. Vishal Vijayan (2015) recorded the highest germination percentage in rice, when field soil is mulched with dry leaves of Acacia.

Lowering the concentration of allelochemicals induce more stimulation in plant growth. It improves cell division and cellular regulation under chilling conditions to acclimate the plant roots. Maqbool *et al.* (2012), found that the *Galinsoga parviflora* water extracts at low concentration improved chilling resistance of *Vicia faba*. Phiri (2010) found that the Moringa water extract increased sorghum germination, maize radical length and hypocotyl length when applied on plant foliage at low concentration. Maqbool *et al.* (2012), reported that low concentrations of allelopathic water extracts as seed treatment before sowing or planting can improve germination percentage, germination power, germination index, radical length, plumule length, fresh weight and dry weight of plants. The inhibition of seedling length and biomass may be due to the presence of higher amount of volatiles, chemicals or phenolic compounds. The present study support the earlier record by del Moral and Muller (1970).

According to Rice, (1974) the visible effects of allelochemicals on the growth and development of plants include inhibited or retarded germination rate; seeds darkened and swollen; reduced root or radicle and shoot or coleoptiles extension; swelling or necrosis of root tips; curling of the root axis; discoloration, lack of root hairs; increased number of seminal roots; reduced dry weight accumulation; and lowered reproductive capacity. These gross morphological effects maybe secondary manifestations of primary events, caused by a variety of more specific effects acting at the cellular or molecular level in the receiver plants.

Bertin *et al.* (2003); Crist and Sherf (1973); Willis (2000) found that the members of Juglandaceae family produce juglone (5-hydroxy-1, 4-naphthoquinone) which is a potent allelochemical that can inhibit the growth of a large number of plants at concentrations as low as 1  $\mu$ M. Sensitive plants such as herbaceous and woody species of tomato, potato, apple, cucumber, watermelon, alfalfa, wheat, corn etc. that can exhibit wilting, browning of vascular tissues, necrosis and eventually death when cultivated close to established black walnut trees.

The higher degree of adverse effect was observed in ragi treated with Teak 50% concentration of leaf extract followed by 30, 20, 10 and 5%. The results of GC-MS analysis showed the presence of terpenoids such as sesquiterpenoids, diterpenes and triterpenes, n-Hexadecanoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 9,12,15-Octadecatrienoic acid; Phytol and Spathulenol formic acid, 2-pentanone, propanoic acid, butanoic acid, limonene oxide, 1, 2-propanediol, 2-acetate, propanoic acid, methyl ester, phenol, glycerine, butanol, benzofuran, propanal, butanal, acetic anhydride etc. in Teak.

The chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents under Teak leaf extract treatments are given in the Fig :3 and 4. The highest decreasing percentage of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid was noticed at 50% in ragi and pearl millet when compared with 5%, 10%, 20%, 30% concentration of leaf extract and control seedlings. In all the extract treatments, ragi and pearl millet showed more reduction percentage in chlorophyll-a than chlorophyll-b. But in pearl millet, less reduction percentage of carotenoid was observed in all treatments compared to ragi. Decreasing trend on pigment content was recorded in the test crops with increasing concentrations (10, 20, 30 and 50%) of leaf extract of Teak. The effect might be due to degradation of chlorophyll pigments or reduction in their synthesis and the action of flavanoids, terpenoids or other phytochemicals present in leaf extract (Tripathi *et al.*, 1999, 2000). The more reduction of chlorophyll-a than chlorophyll-b, indicate its susceptibility to stress (Djanaguiraman *et al.*, 2003). During stress situation, in tolerant species conversion of chlorophyll-a to chlorophyll-b may occur (Djanaguiraman *et al.*, 2003). At higher concentrations allelochemicals may act as photosynthetic inhibitors which block electron acceptors, act as energy uncouplers and reduce the activity of photosynthetic pigments and enzymes (Einhellig and Rasmussen, 1979). However, a positive role can be predicted at their lower concentrations. Growth is promoted through optimum CO<sub>2</sub> fixation under normal conditions at relatively low concentrations of secondary metabolites.

The impact of phenolic allelochemicals on the respiration of plants has mainly been shown to involve weakened oxygen absorption capacity, while the impact on photosynthesis has mainly been to reduce the chlorophyll content and photosynthetic rate. Phenolic allelochemicals can also lead to increased cell membrane permeability. It inhibits plants from absorbing nutrients from surroundings and affect the normal growth of plants. Patterson (1981) reported that

10-30  $\mu$ mol/L caffeic acid coumaric acid, ferulic acid, cinnamic acid and vanillin could significantly inhibit the growth of soybean.

The highest inhibitory effect was found in ragi at 50% concentration of Teak leaf extract. It may be due to their high concentration of phenol content along with other constituents in the extracts. The phenolic compound might have interference with phosphorylation pathway or inhibiting the activation of Mg<sup>2+</sup> and ATPase activity or might be due to decreased synthesis of total carbohydrate, protein and nucleic acid (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes (Pawar *et al.*, 2004). Abu-Romman (2011) reported that photosynthetic pigments in *Capsicum annum* seedlings were significantly and negatively affected by treatment with *Achillea biebersteinii*.

Fig-5 shows the starch, protein, amino acid and catalase content of the test crops. The higher amount of starch, protein, amino acid and catalase were observed in 5% concentration of extract treated seedlings of ragi and pearl millet over control. When increasing the leaf extract concentrations (10, 20, 30 and 50%) there was a decreasing trend of starch, protein, amino acid and catalase contents both in ragi and pearl millet seedlings. The 50% concentration of leaf extract showed more retarding effect on amino acid content of test crops than starch and protein. In ragi, more protein content was observed than starch and amino acid contents in all treatments. As the chlorophyll concentration decrease in all concentration of extract, the metabolite of starch, protein, amino acid and catalase decreased. Tripathi *et al.* (1998) reported that the lower concentration of leaf extracts of *Acacia nilotica*, *Tectona grandis* and *Albiia procera* showed stimulatory effect on starch, protein and amino acid contents of soybean. But in higher concentration of leaf extract, there was a decreasing trend of these biochemical constituents as observed in the soybean.

Allelochemicals enter through the plant cell membrane and the activity and function of enzymes. Results of other studies reported that root length, shoot length no. of leaves and fresh weight of maize seedlings were significantly reduced after a 6-day treatment with ferulic acid (Devi, 1992). Batish *et al.* (2008) reported that activity of enzymes are significantly reduced in mung bean when treated with 1mM caffeic acid. The decreasing content of biochemical contents may be due to action of phyto pinene, camphene, phenolic aglycons, flavanoids, trans-pinocarveol, limonene oxide, cis, 5,7-octadien-2-ol, 2,6-dimethyl, etc. The combination of different phenolic compounds showed a greater inhibition effect than the individual phenolic acids, which is present in the Teak leaf extracts. The allelochemicals of teak significantly reduced the chlorophyll, carotenoid, starch, protein, amino acid and catalase contents of seedlings. Kohli (1990) reported, that the enzymes like protease, polyphenol oxidase, peroxidase,  $\alpha$ -amylase and  $\beta$ -amylase are affected by the allelochemicals.

## CONCLUSION:

The present investigation revealed that aqueous leaf extracts of Teak at different concentration levels inhibited seedling growth and at low concentration (5%) stimulated the germination, seedling length, biomass, pigments, starch, protein and amino acid contents of ragi and pearl millet seedlings. Inhibitory effect of different concentrations of extract was not equal and highest inhibition was observed in ragi while the lowest inhibition was observed in pearl millet. In both the test crops the promotory effects were observed at 5% concentration of extract. The inhibitory and stimulatory effects of *T. grandis* leaf extracts on ragi and pearl millet may be due to the presence of allelochemicals in the extracts.

Several Agroforestry trees of allelopathy have already been studied and some studies are in progress although some trees are needed to be studied extensively to implicate the mechanism of allelopathy successfully. Further field study must be carried out to exploit the allelopathic potentiality of Teak on field crops using Teak bark and root extracts.

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